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Our aim was to confirm our hypothesis that chronic lithium treatment predisposes to heat-induced illness using an animal model. We hypothesized that lithium-treated rats would develop heat-induced illness more easily and rapidly than would controls. Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. In some experiments rats were offered water *ad libitum*, in other experiments fluid was restricted from day 14-20. During passive heat testing, rats were housed in a small environmental chamber (modified tissue culture incubator) in individual containers. Temperature in the chamber was maintained at 41.5° C with forced hot air, relative humidity at 30-50%. During active (exercise-induced) heat testing, animals were exercised on a motor-driven treadmill in chambers heated with forced hot air to a temperature of 26° C. Humidity was maintained at 30% relative humidity. Lithium treatment did not affect body water distribution, the rate of body temperature rise in either heating model, and the organ damage caused by exposure to heat (as assessed by leakage of enzymes into blood). There was a difference in the tail temperature during cooling after active (exercise-induced) heating - tail temperatures were consistently higher in lithium treated rats. We also observed that lithium treated animals consumed more water when it was offered *ad libitum*.

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Introduction:

Our aim was to confirm our hypothesis that chronic lithium treatment predisposes to heat-induced illness using an animal model. We hypothesized that lithium-treated rats would develop heat-induced illness more easily and rapidly than would controls. Heat-induced illness is a serious medical emergency. Mortality rates of 17-70% are reported, and in the United States more than 4000 people per year die of heatstroke (1). Heat-associated decrements in performance, and heat-injury are serious problems for the military, as strenuous physical activity in hot climates must be required of soldiers in the field. Heat-induced illness is being seen with increased frequency in healthy civilian populations as jogging becomes more prevalent (2).

There are differences between those individuals who develop heatstroke and those who do not. Factors that have been thought to predispose to heat-induced illness include amount of exertion, prior conditioning, pre-existing cardiovascular disease, diabetes mellitus, impairment of sweating due to pharmacologic agents or sweat gland disease, potassium deficiency, and lithium treatment (1-7). Lithium administration has been reported to result in altered distribution of body water, sodium depletion, potassium wasting, polyuria, and abnormal thermoregulation (2,3,8-17). Each of these effects may predispose to developing heat-induced illness.

<u>The rat model of heat stroke</u>

The high rate of mortality precludes the use of humans for controlled studies of the pathophysiology of heat stroke. Therefore suitable animal models are required. Hubbard and colleagues have worked extensively with rats and have found it to be a useful model (18,19-25). Rats are small and inexpensive, and have been extensively studied for decades. We have a large foundation of information about the biology of the laboratory rat which allows us to make predictions about the cardiovascular, behavioral, and metabolic responses to heat stress. Finally, the rat is available in genetically controlled lines, making it possible to expect similar biological responses from different animals. This homogeneity does not exist for sheep or dogs (26,27).

The major mechanism for cooling in the rat is by an increase in blood flow to the tail (23). This is equivalent to the increase of skin blood flow in the human, and allows us to manipulate radiant heat loss (R). The major difference between the rat and the human is the mechanism used to mediate evaporative cooling. The rat does not sweat, but rather secretes copious amounts of saliva, with which it wets its surface and thereby loses heat by evaporation (E). Saliva production by the submaxillary gland is proportional to ambient temperature and core body temperature (23). This cooling mechanism involves behavioral and physiological functions which are different from those involved in sweating (23). This is a problem common to all animal models. Dogs, sheep, ferrets and cats cool themselves via panting. Evaporation during panting cools blood vessels feeding the brain, thereby lowering brain temperature. This mechanism differentially changes brain versus core temperature, and is very different from that which occurs in man. The licking and wetting cooling mechanism of the rat is far closer to the sweating that occurs in man. The average rate of water loss due to salivation and wetting in the rat is similar to the rate of sweat loss in unacclimatized man in heat (23).

Hubbard and colleagues (18, 19-25) have proved that a syndrome similar to human heatstroke can be induced in rats. Hyperthermia and exhaustive exercise result in cell damage. Increased alanine aminotransferase activity in serum (presumably released from damaged liver cells) has been related to the duration and extent of prior hyperthermia (21). Creatine phosphokinase activity in serum (presumably released from damaged muscle cells) increases in proportion to duration of exercise, and is less responsive to hyperthermia (21). Alkaline phosphatase activity in blood is produced by liver, bone and intestine (38), and can be used to detect cell damage within these organs.

Lithium and thermoregulation

Lithium treatment has been reported to alter thermoregulation in man and the rat. In lithium intoxication, body temperature is frequently markedly elevated, with temperatures exceeding 40.8°C (2,3,11,28). Many of the symptoms of heat stroke are similar to those seen in lithium intoxication (3). In psychiatric patients, lithium treatment (not intoxication) results in consistently elevated body temperature throughout the 24 hr circadian temperature rhythm (11). In rats, lithium administration causes an acute hypothermia, but within 3 hours causes significant hyperthermia (body temperature increases of more than 1°C) (11,14). Lithium reverses the hypothermia associated with treating humans with electroshock therapy (28).

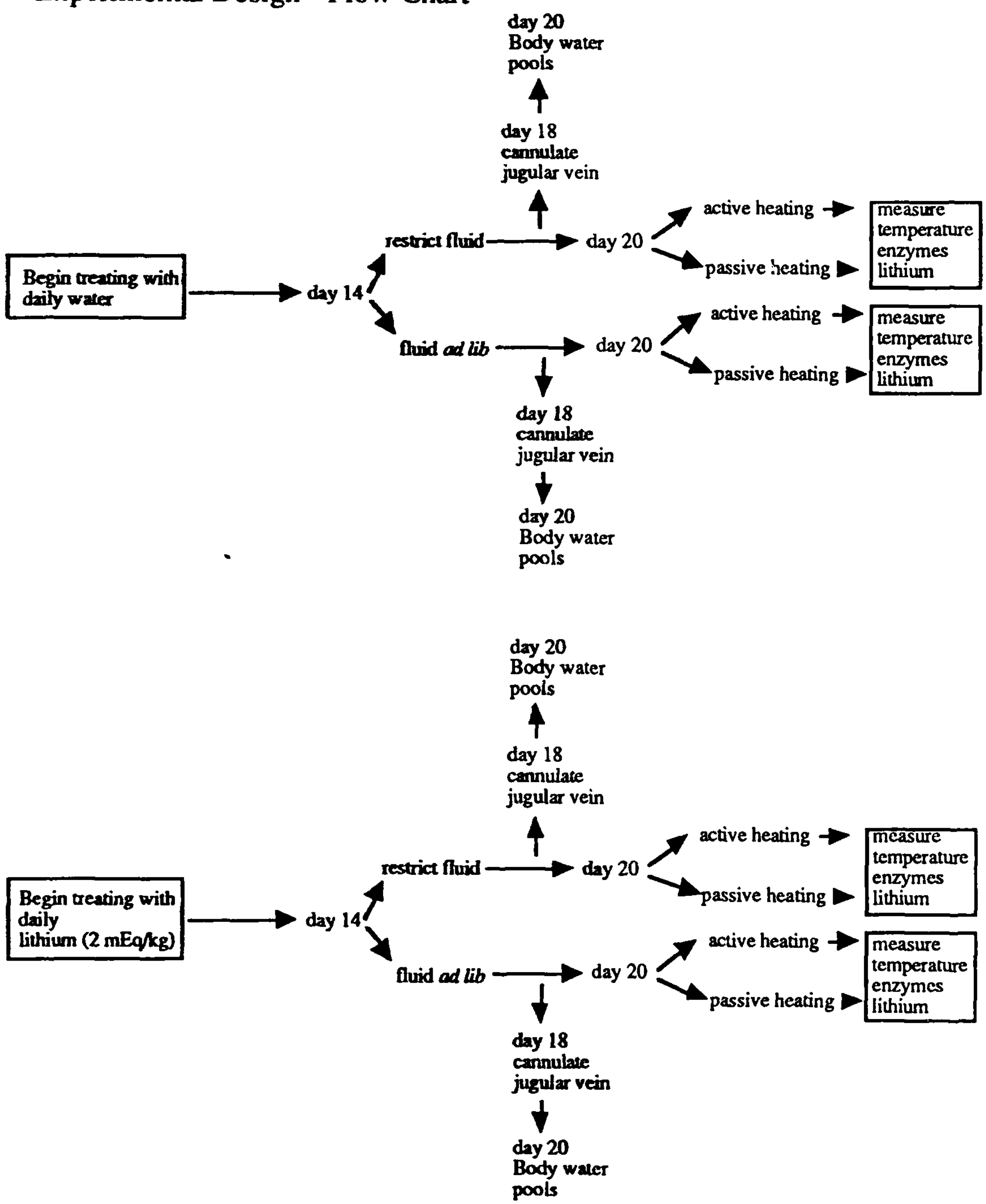
Lithium treatment may make humans more susceptible to heat stress. Individuals treated with lithium prior to marathon races have developed acute heat stroke (2). Rats treated with lithium for as little as 4 days develop fatal hyperpyrexia (core temperature increases more than 3°C) when treated with tranylcypromine, an MAO inhibitor. Tranylcypromine treatment alone, or in combination with pretreatment with sodium, potassium or rubidium was not associated with hyperpyrexia (12,29,30).

BODY

Experimental Methods

Figure 1:

Experimental Design - Flow Chart



Protocol design:

In all studies rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water (1 ml water/100 g body weight), for 20 days. In some experiments rats were offered water *ad libitum*, in dose-ranging experiments water was

restricted from day 14-20 so that 50 ml, 25 ml or 15 ml of water was offered each AM. Once we had established that 15 ml water restriction would be tolerated by rats, this amount of water restriction was compared to no restriction in studies on body water pool sizes and on the effects of passive and active (exercise-induced) heating (see figure 1).

Animals: Male Sprague-Dawley rats, weighing 325 g,were used in all experiments.They were caged individually, in suspended stainless steel wire cages, exposed to light from 0800-1800 daily, in a climate-controlled room (26° C, 30% relative humidity). They were fed a nonpurified diet *ad libitum*, (Charles River Chow #5001, Purina Mills, St. Louis, MO; 23% protein, 4.5% fat, 72% carbohydrate) and water *ad libitum* (except as noted).

Passively induced heat-illness: During heat testing, rats were housed in a small environmental chamber (modified tissue culture incubator) in individual containers. Temperature in the chamber was maintained at 41.5° C with forced hot air, relative humidity at 30-50%.

Exertion induced heat-illness: During heat-testing, animals were exercised on a motor-driven treadmill. It consisted of a wide belt on metal rollers. One roller was driven at a selected rate by a variable speed motor. A Lucite™ box, partitioned into individual compartments 30 cm long x 10 cm wide, was suspended over the belt, providing a separate running area for each rat. A shock grid was located at the rear of the compartments and the animals learned to avoid being shocked by keeping pace with the belt. The large stainless steel chamber in which the Lucite™ chambers were housed was heated with forced hot air to a temperature of 26° C. Humidity was maintained at 30% relative humidity. Animals were run at 11 m/min at a 6° incline until hyperthermic exhaustion (rectal temperature 42.3°C, animal unable to right itself). This method has previously been used by Hubbard and colleagues (18,19,20).

Temperature measurement: Rectal temperature was monitored using a thermocouple (YSI model #423) placed 6.5 cm into the rectum. Tail temperature was measured using a thermocouple taped to the tail.

Venous catheterization:

Jugular veins were cannulated using a method which has been published previously (31). Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Cannulas (Silastic™ tubing, .020 in. ID, .037 in. OD, Dow Corning) were inserted into the right jugular vein. To prevent slippage, a small amount of silicone adhesive (Medical Adhesive Silicone Type A, Dow Corning) was placed on the cannula at a distance relative to the size of the animal (the end of the cannulas just reached the superior vena cava). A silk ligature is run through the silicone and tied to the vessel. The cannula was exteriorized between the shoulder blades and flushed daily with heparinized saline (10 units/ml). Animals recuperated for approximately 48 hours prior to use.

Blood to be analyzed for enzyme activities was withdrawn immediately before animals were sacrificed, and therefore did not effect body water status during heating. For measurement of body water distribution 1.85 ml of blood/rat was withdrawn for the total study (265 µl per point).

Determination of Total Body Water, Extracellular Fluid Volume and Plasma Volume:

Body water distribution was determined on day 20. Tritiated water (0.5 µCi in 200 µl 0.9% NaCl) was administered intravenously. Ninety minutes later blood was withdrawn from the intravenous catheter (after first withdrawing and discarding contents of the intravenous line) and used to determine total body water. A single 1 cc bolus of ^{14}C-inulin (0.5 µCi) mixed with indocyanine green (1.6 mg) was administered intravenously. Six

blood samples (250µl) were withdrawn at 3,5,7,30,60,and 90 minutes after the dose. This method permitted us to assess extracellular and plasma volumes simultaneously. This technique also eliminated the withdrawal of excessive amounts of blood. Intracellular fluid was determined by subtracting extracellular fluid from total body water. These methods are modifications of those previously described (32,33). Animals used for body water determinations were separate animals from those used for heating experiments. However, these rats were treated identically (in parallel) to the heated groups of animals for the first 20 days of the studies.

Alkaline Phosphatase:

Alkaline phosphatase activity in plasma was assayed using the method of Bessey, *et al.* (34). The assay was based upon the conversion of p-nitrophenyl phosphate to p-nitrophenol, which formed a colored complex at basic pH. Absorbance at 410 nm was monitored and we calculated enzyme activity by extrapolating from a calibration curve obtained from using diluted *p*-nitrophenol and 0.02N NaOH.

Alanine Aminotransferase (ALT):

Alanine aminotransferase activity in plasma was measured based upon the assay of Bergmeyer *et al.* (35). The assay was based upon the conversion of L-alanine + 2-oxoglutarate to pyruvate + L-glutamate. The pyruvate, in the presence of NADH and lactic dehydrogenase formed NAD and lactate. Absorbance at 340 nm was monitored and we calculated enzyme activity using change in absorbance/min.

Creatine Phosphokinase (CPK)

CPK activity in plasma was measured using the assay of Okinaka *et al.* (36). The assay was based upon the following enzymatic reactions:

$$\text{ATP + Creatine} \xrightarrow{\text{CPK}} \text{ADP + Phosphocreatine}$$

$$\text{Phosphocreatine} \xrightarrow{\text{hydrolysis}} \text{Creatine + Pi}$$

$$\text{Pi + Acid Molybdate} \longrightarrow \text{Colored Complex}$$

Absorbance was monitored at 660 nm and we calculated enzyme activity by extrapolating from a calibration curve obtained using inorganic phosphate.

Lactate Dehydrogenase (LDH)

LDH activity was measured in serum using the method of Cabaud and Wroblewski (37). The assay was based upon the following enzymatic reactions:

$$\text{Pyruvic Acid + NADH} \xrightarrow{\text{LDH}} \text{Lactic Acid + NAD}$$

$$\text{Remaining pyruvate + 2,4-dinitrophenylhydrazine} \longrightarrow \text{hydrazone}$$

$$\text{hydrazone + NaOH} \longrightarrow \text{colored complex}$$

Absorbance was monitored at 450 nm and we calculated enzyme activity by extrapolating from a calibration curve obtained using known amounts of pyruvate and 2,4-dinitrophenylhydrazine.

Measurement of Plasma and Tissue Lithium Concentrations:

Total lithium in plasma was measured by atomic absorption spectrophotometry using a Perkin Elmer Zeeman 5000 Atomic Spectroscopy System calibrated to a 1 ppm lithium standard.

RESULTS

Body weight:

Lithium treated animals gained the same amount of weight as did controls (figures 2-7). Each data point represents at least n=5.

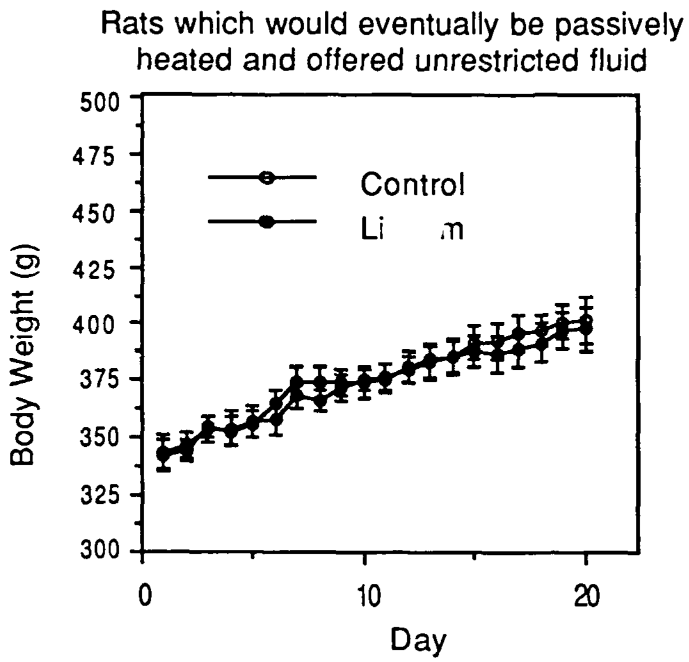


Fig. 2: Weight gain of rats which would eventually be passively heated and offered unrestricted fluid.

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* . Data are presented as mean weight ± standard error of the mean.

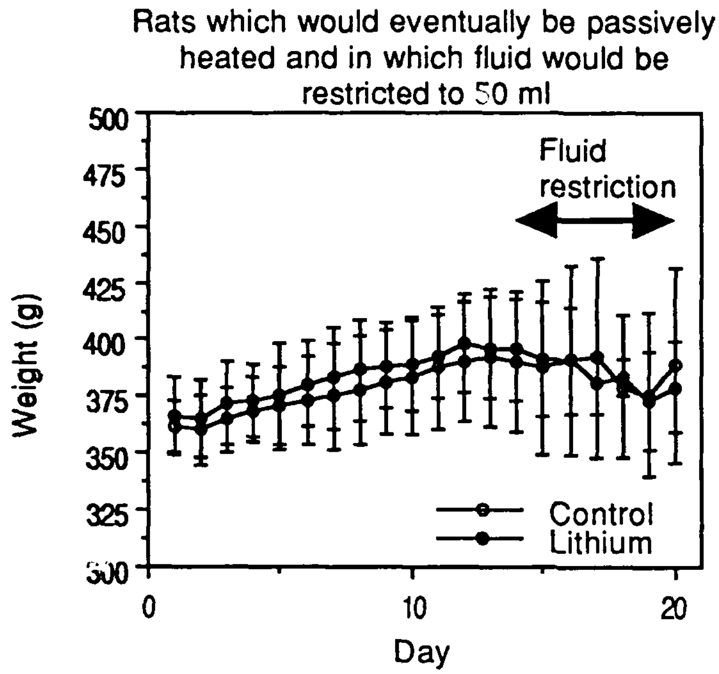


Fig. 3: Weight gain of rats which would eventually be passively heated and offered restricted fluid (50 ml/day for last week).

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* until day 14 when they were restricted to 50 ml of water per day. Data are presented as mean weight ± standard deviation of the mean.

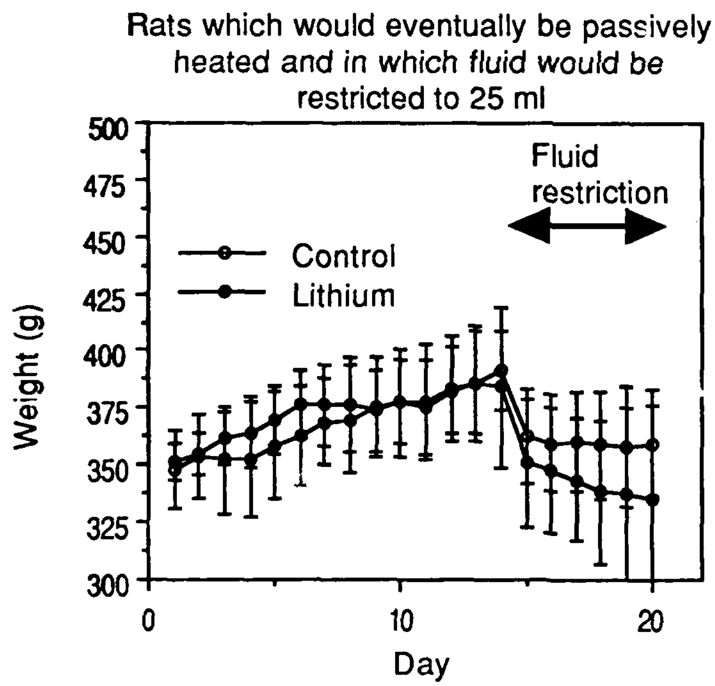


Fig. 4: Weight gain of rats which would eventually be passively heated and offered restricted fluid (25 ml/day for last week).

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* until day 14 when they were restricted to 25 ml of water per day. Data are presented as mean weight ± standard deviation of the mean.

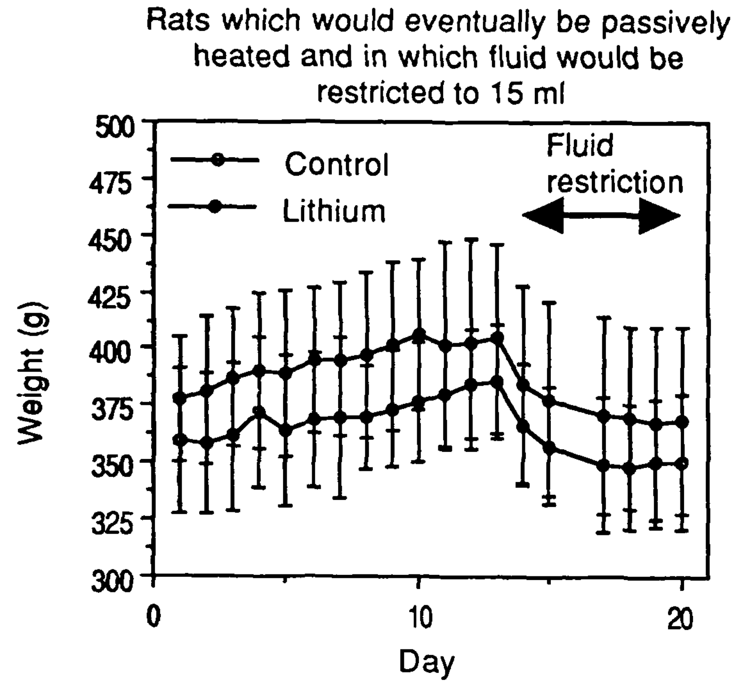


Fig. 5: Weight gain of rats which would eventually be passively heated and offered restricted fluid (15 ml/day for last week).

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* until day 14 when they were restricted to 15 ml of water per day. Data are presented as mean weight ± standard deviation of the mean.

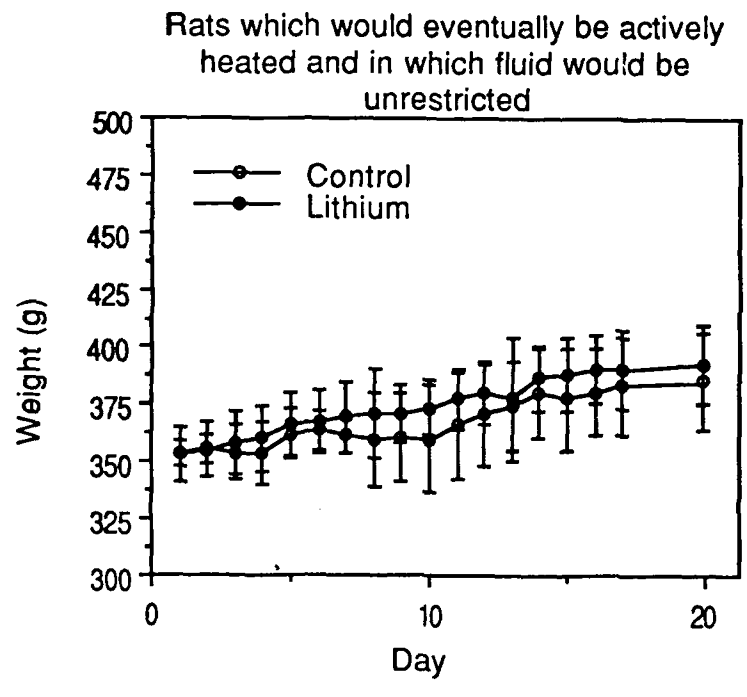


Fig. 6: Weight gain of rats which would eventually be active (exercise-induced) heated and offered unrestricted fluid.

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* . Data are presented as mean weight ± standard deviation of the mean.

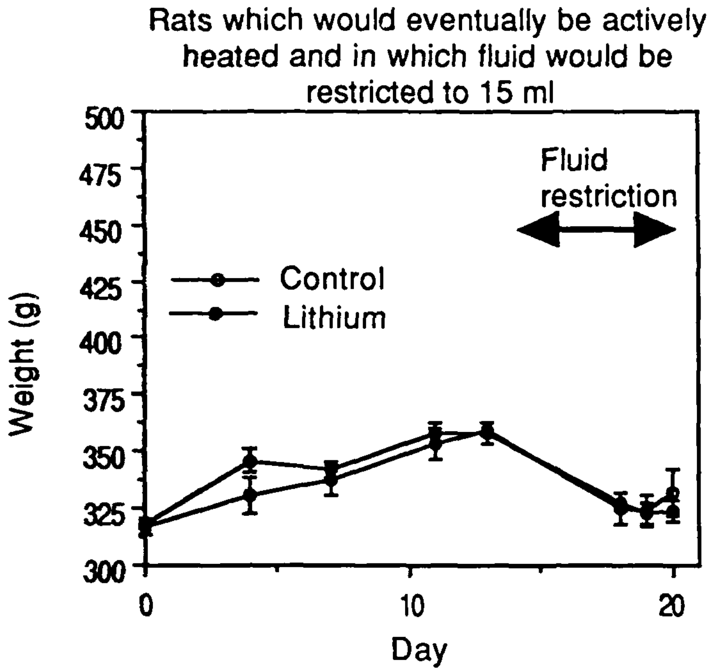


Fig. 7: Weight gain of rats which would eventually be active (exercise-induced) heated and offered restricted fluid (15 ml/day for last week).

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* until day 14 when they were restricted to 15 ml of water per day. Data are presented as mean weight ± standard deviation of the mean.

Water Intake

On unrestricted access to water, water intakes by lithium treated animals on days 7,8,9,10,11 and 15-20 are $p < 0.05$ different from those of controls by 2-way ANOVA (figure 8).

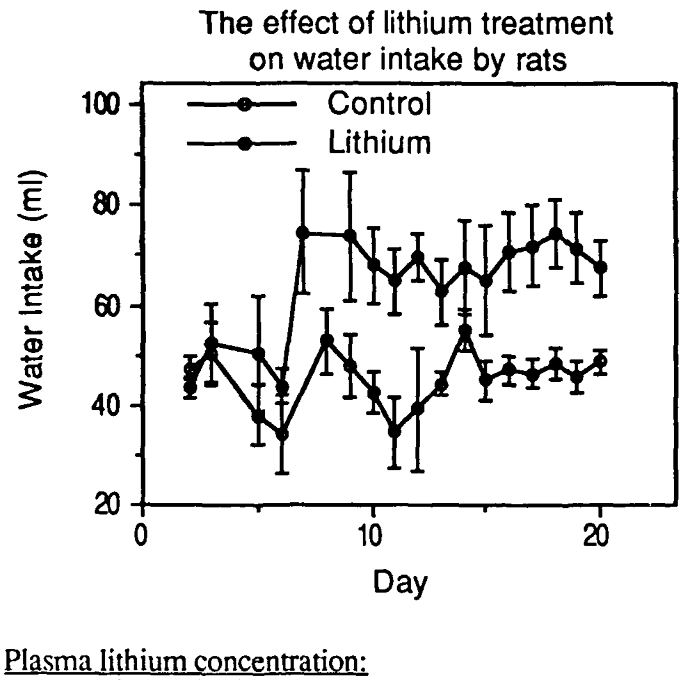


Fig 8: The effect of lithium treatment on water intake by rats

Rats were treated with lithium (2 mEq/kg body weight/day), or with a matching amount of water, for 20 days. They were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum*. Data are presented as mean ± standard error of the mean.

Plasma lithium concentration:

Plasma lithium concentration rose rapidly after a single oral dose of 2 mEq/kg, attaining concentrations of 0.3-0.4 mM within an hour. After repeated doses (administered 2 mEq/kg daily) plasma rose to 0.5 to 0.6 mM. (see figure 9 & 10).

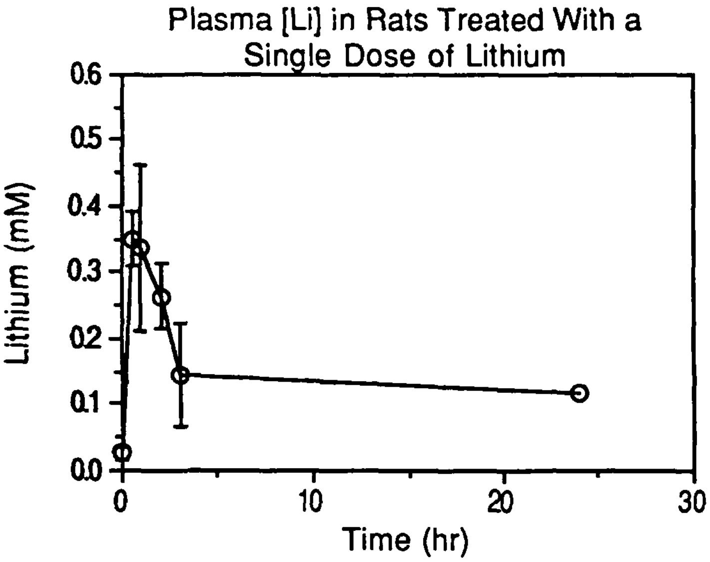


Fig 9: Plasma lithium concentration after a single dose of lithium.

Rats were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum*. At time 0, they were treated orally with lithium (2 mEq/kg body weight). Blood was drawn by cardiac puncture into a heparinized syringe. Plasma lithium levels were measured by atomic absorbance spectrometry. Data are presented as mean ± standard deviation of the mean.

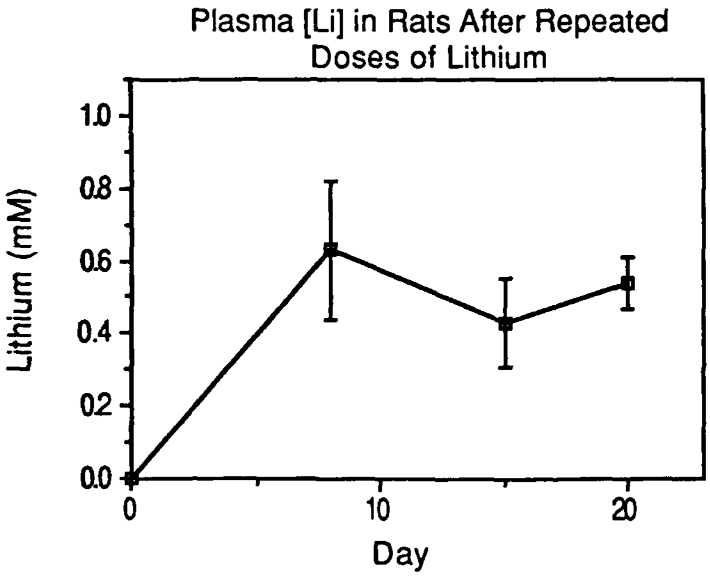


Fig 10: Plasma lithium concentration after repeated doses of lithium.

Rats were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum*. Rats were treated orally with lithium (2 mEq/kg body weight/day) for the number of days indicated. Blood was drawn by cardiac puncture. Plasma lithium levels were measured by atomic absorbance spectrometry. Data are presented as mean ± standard deviation of the mean.

Body water distribution:

Total body water was significantly decreased by fluid restriction ($p<0.05$ in lithium treated groups, $p < 0.01$ in controls by 1-way ANOVA and Scheffe's test) but was not influenced by lithium treatment (figure 11). Extracellular fluid volume was significantly decreased by fluid restriction ($p<0.05$ in lithium treated groups, $p < 0.01$ in controls by 1-way ANOVA and Scheffe's test) but was not influenced by lithium treatment (figure 12). There were no significant differences in intracellular fluid volume (figure 13). Plasma volume was significantly decreased by fluid restriction ($p<0.05$ in controls vs 15 ml controls by 1-way ANOVA and Scheffe's test) but was not influenced by lithium treatment (figure 14).

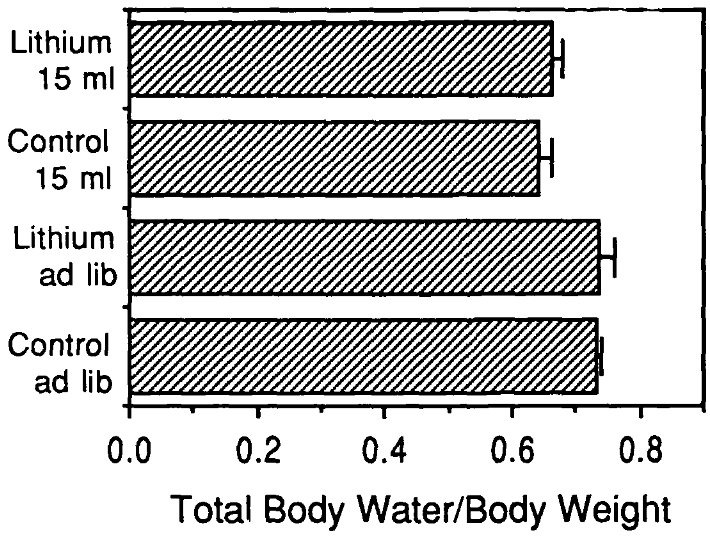


Fig 11: Total body water measurements.

Rats were caged individually and were fed a nonpurified diet *ad libitum* and were offered water *ad libitum* or restricted to 15 ml water/day (as indicated). Rats were treated orally with lithium (2 mEq/kg body weight/day; Lithium groups) or with an equivalent amount of water (Control groups) for 20 days. Total body water was determined as described in text. Data are presented as mean ± standard error of the mean. (n=7 for control *ad lib*, 5 for control 15 ml, 4 for lithium *ad lib*, and 5 for lithium 15 ml).

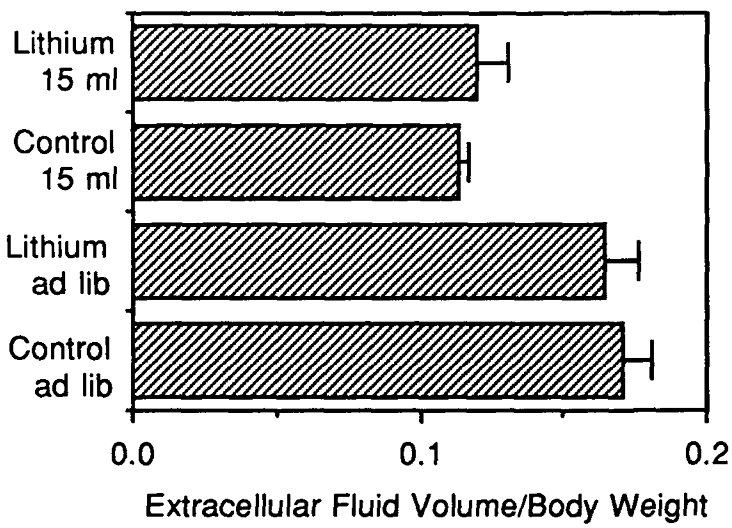


Fig 12: Extracellular body water measurements.

Rats were treated as described in figure 11. Extracellular body water was determined as described in text. Data are presented as mean ± standard error of the mean.

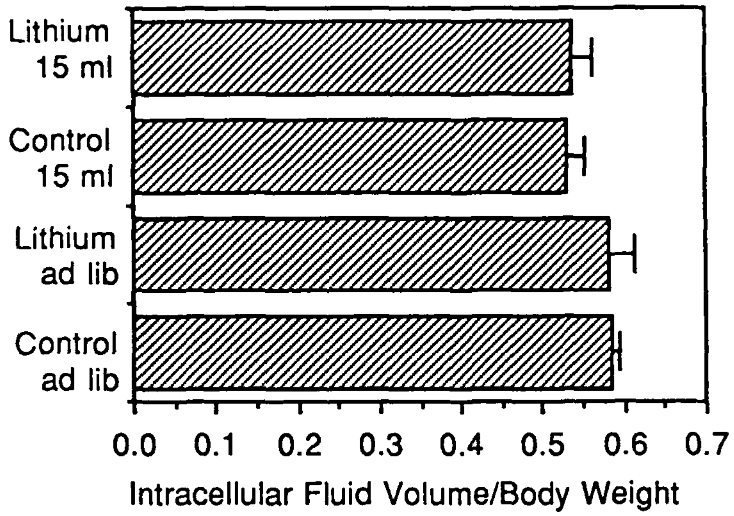


Fig 13: Intracellular body water measurements.

Rats were treated as described in figure 11. Intracellular fluid volume was determined by subtracting extracellular fluid volume from total body water volume. Data are presented as mean ± standard error of the mean.

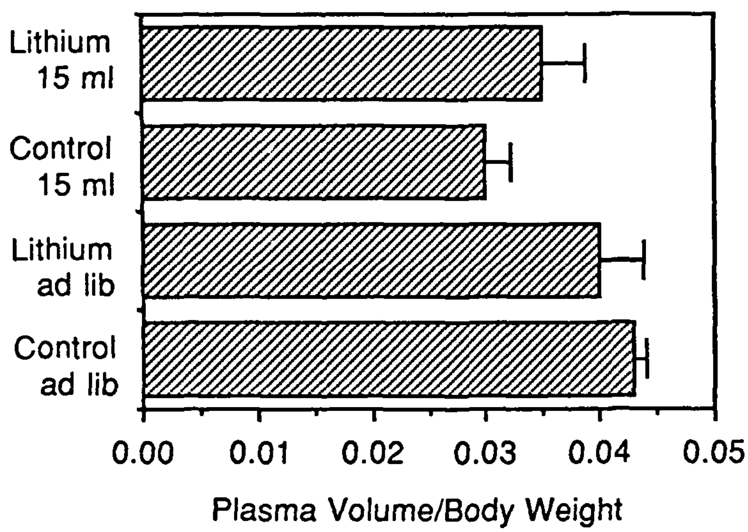


Fig 14: Plasma volume measurements.

Rats were treated as described in figure 11. Plasma volume was determined as described in text. Data are presented as mean ± standard error of the mean.

TABLE 1: Plasma volume (milliliters)

Group	Plasma volume (ml) ± std. error
Control *ad libitum* water	16.96 ± 0.54
Control 15 ml restricted	11.27 ± 0.90
Lithium *ad libitum* water	16.55 ± 1.56
Lithium 15 ml restricted	13.48 ± 1.76

Response to Passive Heating

In rats offered water *ad libitum*, the rise in body temperature during passive heating was identical in controls and lithium treated animals (figure 15). In rats offered water 50

ml/day for the last week of study, the rise in body temperature during passive heating was identical in controls and lithium treated animals (figure 16).

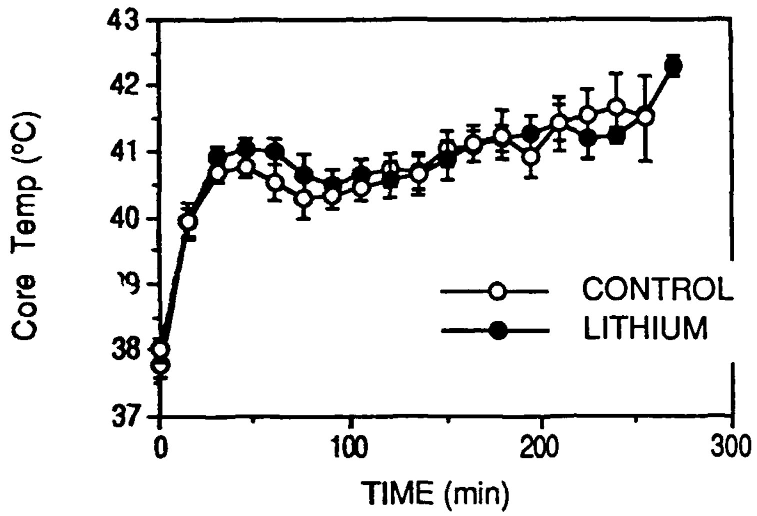


Fig 15: The effect of passive heating on lithium-treated and control rats receiving fluids *ad libitum*. Rats were treated as described in figure 2. Rats were placed in an incubator with an ambient temperature of 41.5°C and 30-50% relative humidity. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. Data are presented as mean ± standard deviation of the mean (n=9 for controls, n=7 for lithium treated).

TABLE 2: Data for figure 15 (means for groups)

Time (min)	Control Core temp(°C)	Std. Dev.	Lithium treated Core temp(°C)	Std. Dev.
0	38.00	0.43	37.80	0.62
15	39.93	0.67	39.93	0.67
30	40.68	0.34	40.91	0.55
45	40.78	0.45	41.03	0.53
60	40.53	0.72	41.01	0.61
75	40.28	0.70	40.63	0.97
90	40.35	0.50	40.49	0.70
105	40.47	0.55	40.65	0.69
120	40.57	0.64	40.71	0.80
135	40.63	0.68	40.69	0.82
150	40.87	0.72	41.05	0.83
165	41.12	0.69	41.09	0.70
180	41.25	0.90	41.19	0.60
195	40.92	0.80	41.29	0.74
210	41.42	1.01	41.42	0.84
225	41.55	0.99	41.20	0.89
240	41.67	1.27	41.23	0.35
255	41.50	1.56	41.55	0.21
270			42.30	0.44

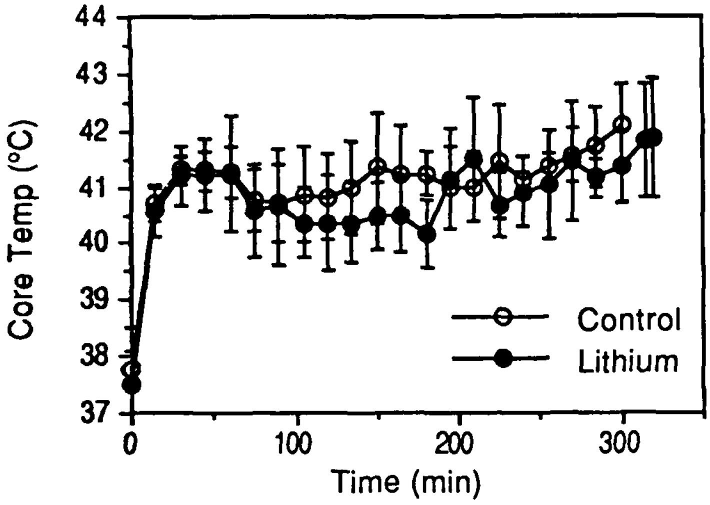


Fig 16: The effect of passive heating on rats restricted to 50 ml of water per day.

Rats were treated as described in figure 3. Water intake was restricted to 50 ml/day for the last week of treatment. Rats were placed in an incubator with an ambient temperature of 41.5°C and 30-50% relative humidity. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. (n=7 for both control and lithium groups, values plotted are mean ± standard deviation of the mean)

TABLE 3: Data for figure 16 (means for groups)

Time (min)	Lithium treated Core temp(°C)	Std. Dev.	Control Core temp(°C)	Std. Dev.
0	37.49	0.60	37.77	0.75
15	40.56	0.47	40.69	0.29
30	41.19	0.53	41.29	0.25
45	41.19	0.64	41.29	0.34
60	41.21	1.03	41.27	0.46
75	40.55	0.82	40.76	0.54
90	40.65	1.02	40.70	0.70
105	40.36	0.62	40.86	0.86
120	40.36	0.83	40.82	0.76
135	40.36	0.69	40.98	0.84
150	40.48	0.59	41.33	0.97
165	40.50	0.68	41.23	0.85
180	40.17	0.59	41.23	0.38
195	41.12	0.89	40.97	0.74
210	41.48	1.08	41.00	0.61
225	40.67	0.58	41.43	1.02
240	40.90	0.62	41.10	0.14
255	41.03	0.95	41.35	0.21
270	41.43	1.04	41.55	0.49
285	41.15	0.35	41.70	0.71
300	41.35	0.64	42.10	0.71
315	41.80	0.99		
320	41.85	1.06		

In rats offered water 25 ml/day for the last week of study, the rise in body temperature during passive heating was identical in controls and lithium treated animals (figure 17).

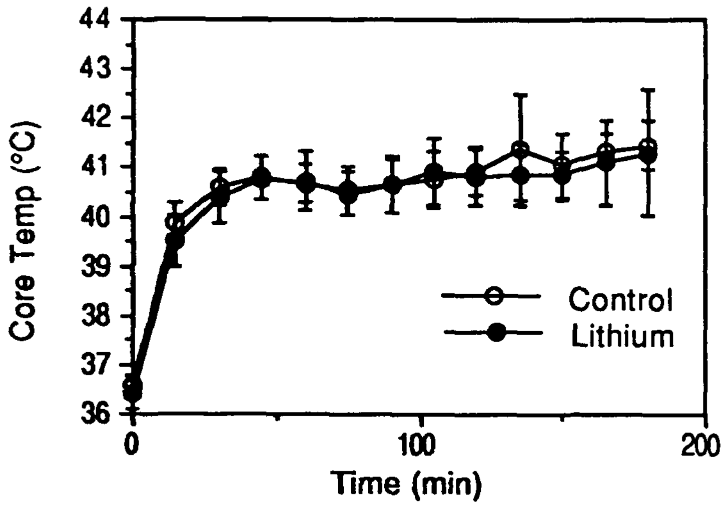


Fig 17: The effect of passive heating on rats restricted to 25 ml of water per day.

Rats were treated as described in figure 4. Water intake was restricted to 25 ml/day for the last week of treatment. Rats were placed in an incubator with an ambient temperature of 41.5°C and 30-50% relative humidity. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. (n=3 for both control and lithium groups, values plotted are mean ± standard deviation of the mean).

TABLE 4: Data for figure 17 (means for groups)

Time (min)	Lithium treated Core temp(°C)	Std. Dev.	Control Core temp(°C)	Std. Dev.
0	36.43	0.35	36.57	0.12
15	39.50	0.52	39.87	0.42
30	40.40	0.53	40.60	0.35
45	40.77	0.45	40.80	0.10
60	40.73	0.61	40.67	0.40
75	40.47	0.46	40.53	0.50
90	40.67	0.57	40.63	0.57
105	40.90	0.70	40.77	0.55
120	40.80	0.57	40.93	0.51
135	40.85	0.64	41.40	1.06
150	40.85	0.49	41.05	0.64
165	41.10	0.85	41.35	0.35

In rats offered water 15 ml/day for the last week of study, the rise in body temperature during passive heating was identical in controls and lithium treated animals (figure 18).

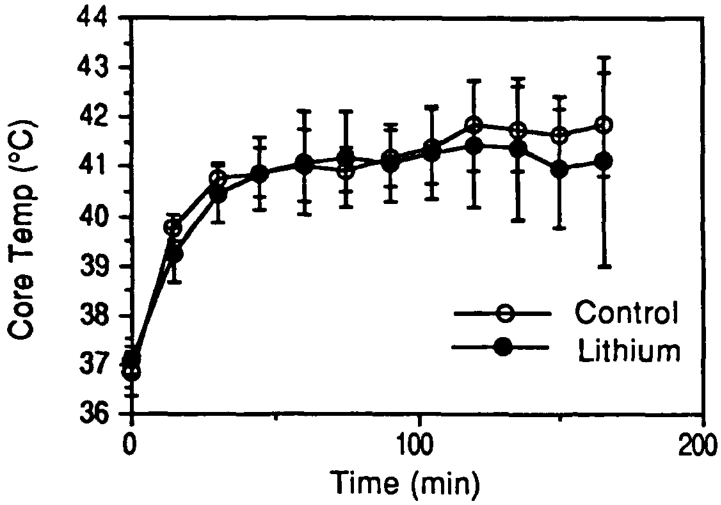


Fig 18: The effect of passive heating on rats restricted to 15 ml of water per day.

Rats were treated as described in figure 5. Water intake was restricted to 15 ml/day for the last week of treatment. Rats were placed in an incubator with an ambient temperature of 41.5°C and 30-50% relative humidity. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. (n=5 for both control and lithium groups, values plotted are mean ± standard deviation of the mean).

TABLE 5: Data for figure 18 (means for groups)

Time (min)	Lithium treated Core temp(°C)	Std. Dev.	Control Core temp(°C)	Std. Dev.
0	37.08	0.27	36.82	0.44
15	39.24	0.55	39.76	0.25
30	40.44	0.58	40.74	0.33
45	40.84	0.73	40.88	0.48
60	41.08	1.06	41.02	0.72
75	41.16	0.98	40.94	0.45
90	41.08	0.78	41.18	0.56
105	41.30	0.94	41.40	0.76
120	41.45	1.29	41.84	0.90
135	41.37	1.43	41.77	0.85
150	40.95	1.20	41.65	0.78
165	41.10	2.12	41.85	1.06

Fluid restriction shortened the time it took for core temperature to reach 42.6°C (from approximately 250 min in unrestricted rats, to approximately 175 min in 15 ml restricted rats).

Response to Active (exercise-induced) Heating

In rats offered water *ad libitum*, the rise in core body temperature (figure 19) and in tail temperature (figure 20) during active (exercise-induced) heating was identical in controls and lithium treated animals. Cooling rate of core temperature was the same in both groups (figure 21), however changes in tail temperature during the 20 minute cooling period were different - control animals consistently had cooler tail temperatures than did the lithium treated animals (figure 22).

In rats offered water 15 ml/day for the last week of study, the rise in core body temperature (figure 23) and in tail temperature (figure 24) during active (exercise-induced) heating was identical in controls and lithium treated animals. Cooling rate of core and tail temperature was the same in both groups (figures 25 and 26).

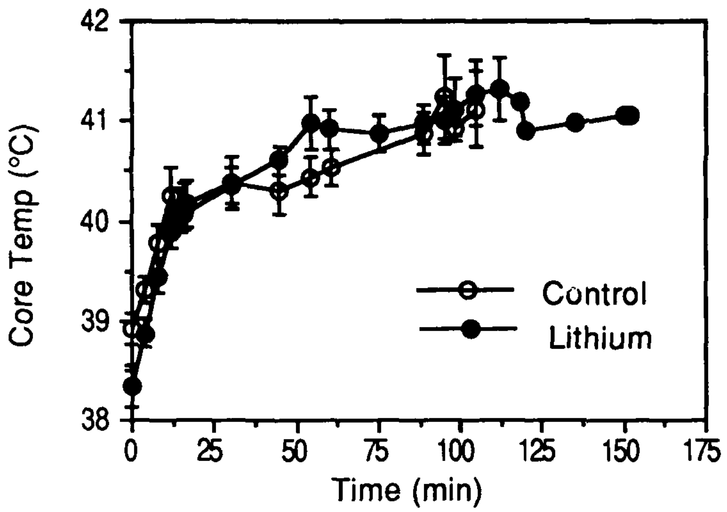


Fig 19: The effect of active (exercise-induced) heating on core body temperature in lithium-treated and control rats offered water *ad libitum*.

Rats were treated as described in figure 6. Rats ran at 6° incline and a pace of 11 m/min, which was maintained by shock avoidance. There was a two minute rest period after 20 and 40 min of work. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace.Core temperatures were monitored continuously throughout the run with a rectal probe. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. Data are presented as mean ± standard error of the mean (n=6 for control and lithium-treated rats).

Note: Data in figure 19 are presented as mean core temperature for groups of diminishing size (until all but 1 rat/group reached 42.6°C). The mean (± standard error of the mean) run time for the all animals in each group were 100.7 min ±14 for controls and 115.7 ± 16.1 min for the lithium treated group.

TABLE 6: Data for figure 19 (means for groups)

Time (min)	Lithium treated Core temp(°C)	Std. Dev.	Control Core temp(°C)	Std. Dev.
0	38.35	0.51	38.92	0.40
4	38.87	0.35	39.31	0.33
8	39.44	0.39	39.79	0.45
12	39.88	0.37	40.26	0.68
13			40.08	0.55
15			40.10	0.56
16	40.07	0.36	40.14	0.56
17			40.17	0.59
30	40.35	0.45	40.38	0.63
45	40.61	0.34	40.31	0.59
54	40.97	0.64	40.44	0.49
60	40.92	0.45		
61			40.54	0.45
75	40.87	0.46		
89	40.97	0.46	40.87	0.41
95	41.00	0.51	41.25	0.59
98	41.11	0.61	40.92	0.12
105	41.28	0.66	41.12	0.54
112	41.32	0.63		
118	41.20	0.58		
120	40.89	0.06		
135	40.99	0.02		
150	41.06	0.04		
152	41.06	0.06		

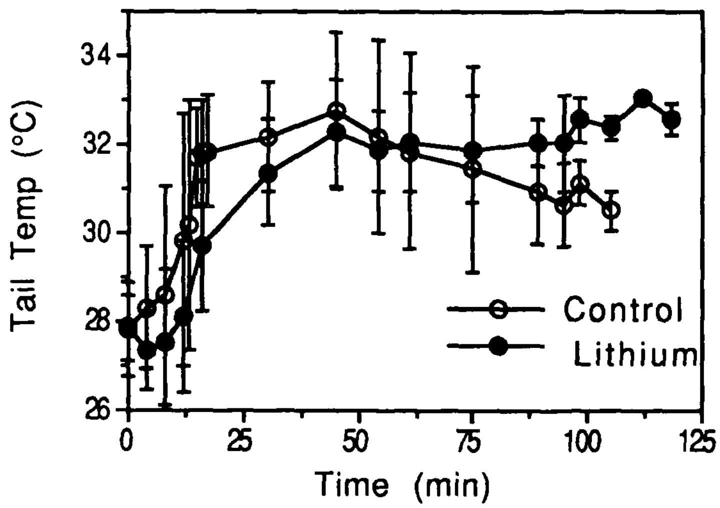


Fig 20: The effect of active (exercise-induced) heating on tail temperature in lithium-treated and control rats offered water *ad libitum*.

Rats were treated as in figure 19 except that tail temperatures were measured with a surface probe attached to the tail. Data are presented as mean ± standard error of the mean (n=6 for control and lithium-treated rats).

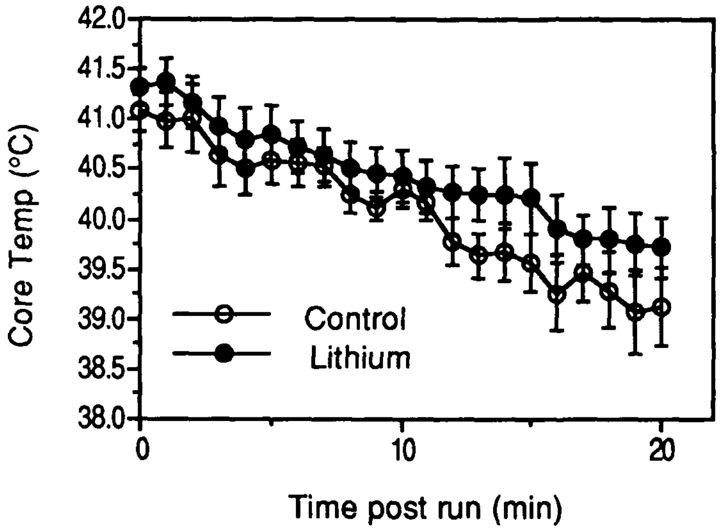


Fig 21: Core temperature after active (exercise-induced) heating in lithium-treated and control rats offered water *ad lib*.

Rats were treated as in figure 19. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace. Animals were then removed from the treadmill and allowed to cool (start of cooling = time 0). Data are presented as mean ± standard error of the mean (n=6 for both groups).

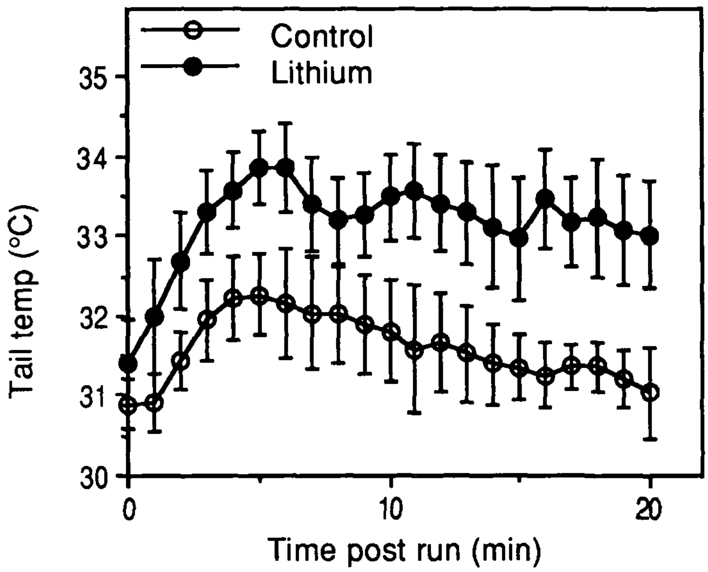


Fig 22: Tail temperature after active (exercise-induced) heating in lithium-treated and control rats offered water *ad lib.*

Rats were treated as described in figure 19. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace. Animals were then removed from the treadmill and allowed to cool (start of cooling = time 0). Data are presented as mean ± standard error of the mean (n=6 for both groups).

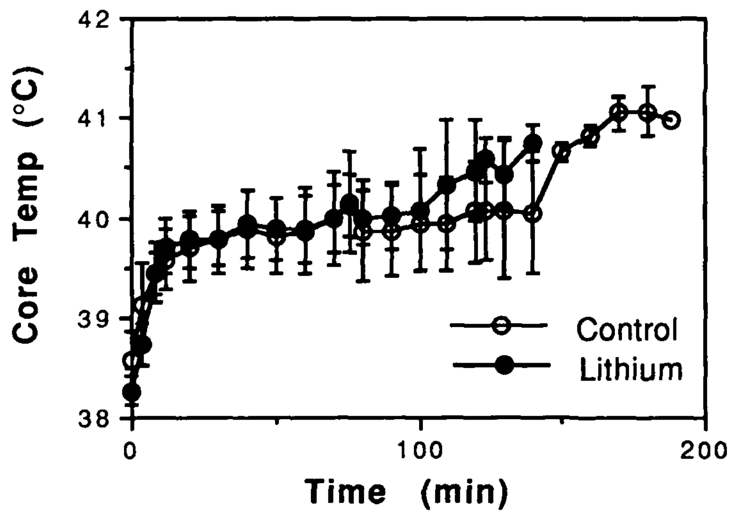


Fig 23: The effect of active (exercise-induced) heating on core body temperature in lithium-treated and control rats offered 15 ml water/day.

Rats were treated as described in figure 7. Rats ran at 6° incline and a pace of 11 m/min, which was maintained by shock avoidance. There was a two minute rest period after 20 and 40 min of work. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace. Core temperatures were monitored continuously throughout the run with a rectal probe. Mean core temperatures are plotted until all but 1 rat/group reached 42.6°C. Data are presented as mean ± standard error of the mean (n=5 and 6 for control and lithium-treated groups, respectively).

TABLE 7: Data for figure 23 (means for groups)

Time (min)	Lithium treated Core temp(°C)	Std. Dev.	Control Core temp(°C)	Std. Dev.
0	38.27	0.35	38.57	0.65
4	38.73	0.52	39.12	0.96
8	39.44	0.51	39.45	0.69
12	39.71	0.65	39.58	0.68
20	39.78	0.68	39.69	0.75
30	39.79	0.68	39.78	0.78
40	39.94	0.83	39.89	0.87
50	39.89	0.76	39.81	0.86
60	39.87	0.77	39.87	0.95
70	39.99	0.75	39.99	1.04
76	40.12	0.68	40.15	1.11
80	40.00	0.53	39.87	1.00
90	40.07	0.86	39.86	0.92
100	40.32	0.91	39.94	0.96
110	40.46	0.71	39.95	0.93
120	40.58	0.33	40.06	1.02
130	40.44	0.52	40.07	1.19
132	40.75	0.26		
140			40.05	1.05
150			40.66	0.13
160			40.82	0.14
170			41.06	0.23
180			41.07	0.35
188			40.98	0.23

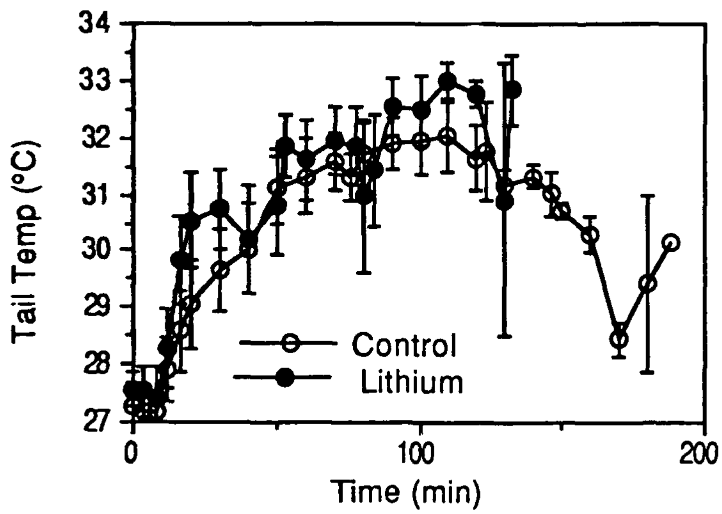


Fig 24: The effect of active (exercise-induced) heating on tail temperature in lithium-treated and control rats offered 15 ml water/day.
Rats were treated as described in figure 23. Tail temperatures were measured with a surface probe attached to the tail. Data are presented as mean ± standard error of the mean (n=5 and 6 for control and lithium-treated groups, respectively).

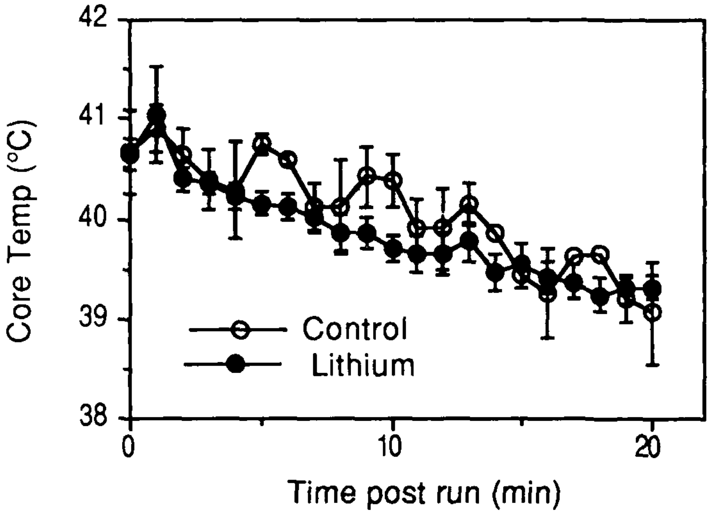


Fig 25: Cooling rate (Core temperature) after active (exercise-induced) heating in lithium-treated and control rats offered 15 ml water/day.
Rats were treated as described in figure 23. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace. Animals were then removed from the treadmill and allowed to cool (start of cooling (end of rune) = time 0). Data are presented as mean ± standard error of the mean (n=5 and 6 for control and lithium-treated groups, respectively).

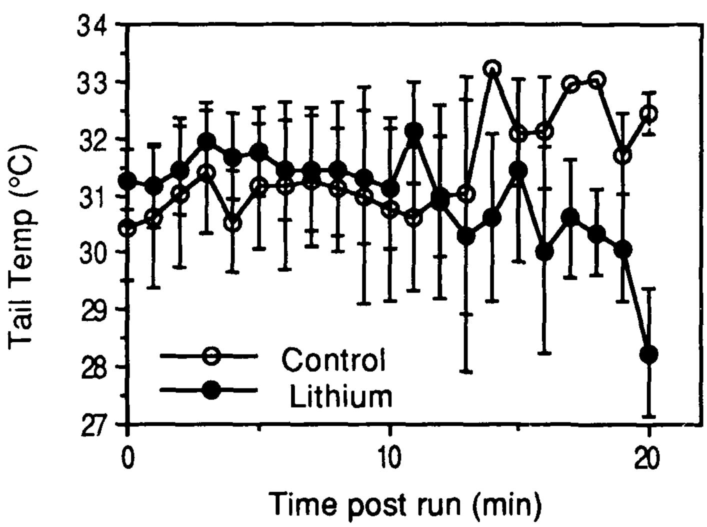


Fig 26: Tail temperature after active (exercise-induced) heating in lithium-treated and control rats offered 15 ml/day water.
Rats were treated as described in figure 23. The end of the active (exercise-induced) heating period was determined as the point of physical exhaustion when the animal would refuse to either right itself or could not keep pace. Animals were then removed from the treadmill and allowed to cool (start of cooling (end of rune) = time 0). Data are presented as mean ± standard error of the mean (n=5 and 6 for control and lithium-treated rats, respectively).

TABLE 8: Weight loss during heating.

Group	Weight loss (% body weight) ± std. error during passive heating	Weight loss (% body weight)± std. error during active heating
Control *ad libitum* water	9.33 ± 1.09	6.56 ± 0.37
Control 50 ml restricted	7.61 ± 1.09	
Control 25 ml restricted	5.84 ± 0.83*	
Control 15 ml restricted	5.18 ± 0.69*	4.04 ± 0.66*
Lithium *ad libitum* water	7.83 ± 1.01	7.10 ± 0.62
Lithium 50 ml restricted	7.33 ± 0.56	
Lithium 25 ml restricted	5.90 ± 1.07*	
Lithium 15 ml restricted	4.85 ± 0.79*	2.76 ± 0.56*

*= $p < 0.05$ different from matched rats given ad libitum water; no differences between lithium and control groups.

TABLE 9: Times to reach 42.6°C and heating rates in response to passive heating.

Group	Rate of weight loss during heating (g/min ± std. error)	Minutes to reach 42.6°C ± std. error	Heating rate °C/min ± std. error
Control *ad libitum* water	0.156 ± 0.005	239 ± 22	0.0260 ± 0.0041
Control 15 ml restricted	0.118 ± 0.006*	155 ± 22*	0.0399 ± 0.0050*
Lithium *ad libitum* water	0.156 ± 0.005	252 ± 21	0.0199 ± 0.0021
Lithium 15 ml restricted	0.124 ± 0.009*	146 ± 25*	0.0436 ± 0.0084*

*= $p < 0.05$ different from matched rats given ad libitum water; no differences between lithium and control groups.

TABLE 10: Weight loss, heating rates and endurance times in response to active (exercise induced) heating.

Group	Rate of weight loss during heating (g/min ± std. error)	Maximum temp. reached (°C) ± std. error	Minutes to reach exhaustion ± std. error	Heating rate °C/min ± std. error
Control *ad libitum* water	0.303 ± 0.029	41.16 ± 0.19	101 ± 14	0.024 ± 0.004
Control 15 ml restricted	0.102 ± 0.021*	40.73 ± 0.38	143 ± 20*	0.017 ± 0.004
Lithium *ad libitum* water	0.267 ± 0.026	41.51 ± 0.21	116 ± 16	0.032 ± 0.007
Lithium 15 ml restricted	0.095 ± 0.012*	40.70 ± 0.16	101 ± 18	0.027 ± 0.003

*= $p < 0.05$ different from matched rats given ad libitum water; no differences between lithium and control groups.

TABLE 11: Degree minutes above 40.5°C

Group	Passive heating (Degree x min ± std. error)	Active heating (Degree x min ± std. error)
Control *ad libitum* water	128 ± 11	26 ± 8
Control 15 ml restricted	117 ± 18	18 ± 10
Lithium *ad libitum* water	124 ± 24	48 ± 14
Lithium 15 ml restricted	96 ± 17	13 ± 8

Changes in plasma activities of alkaline phosphatase, alanine aminotransferase, lactic dehydrogenase and creatine phosphokinase

In rats offered water *ad libitum*, passive and active (exercise-induced) heating increased plasma alkaline phosphatase activity by similar amounts (figure 27; $p<0.01$ vs no heat by 1-way ANOVA and Scheffe's test). In rats restricted to 15 ml water, active (exercise-induced) heating increased plasma alkaline phosphatase activity significantly ($p<0.01$ vs no heat or passive control by 1-way ANOVA and Scheffe's test). Passive heating did not increase plasma alkaline phosphatase activity (figure 28). Passively heated unrestricted animals tended to have higher alkaline phosphatase activity than did passively heated 15 ml restricted rats but this difference was not statistically significant (by 1-way ANOVA and Scheffe's test). There were no effects from lithium treatment.

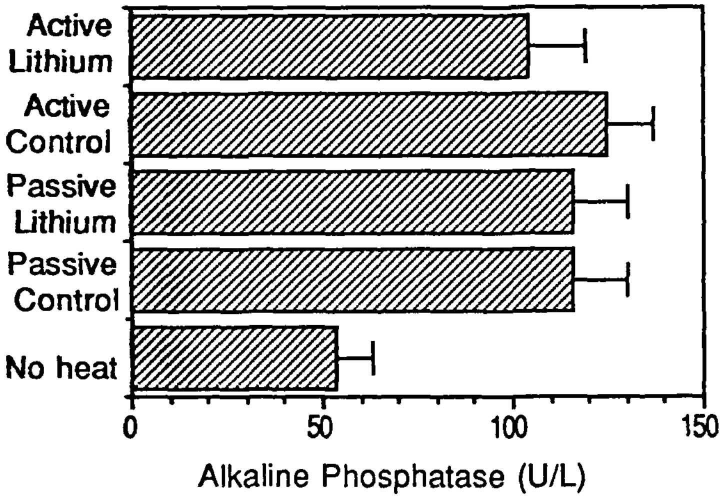


Fig 27: The effect of heating upon plasma alkaline phosphatase activities in lithium-treated and control rats offered water ad lib.
Rats were treated as described in figures 15 and 19. Plasma alkaline phosphatase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) of activity = 1μmole *p*-nitrophenol/hr.

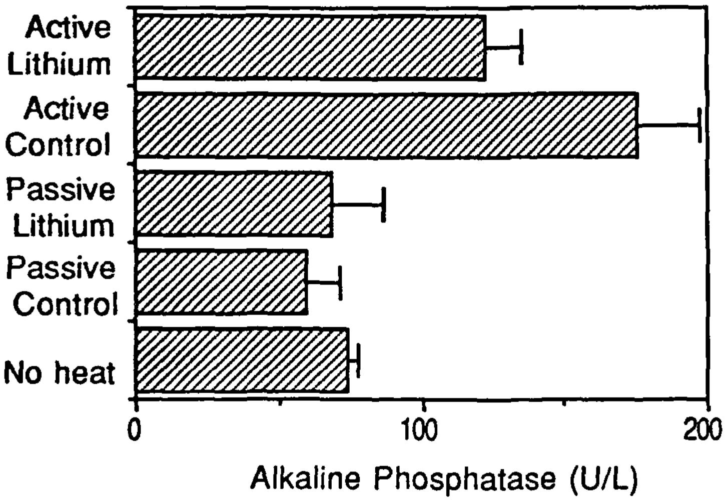


Fig 28: The effect of heating upon plasma alkaline phosphatase activities in lithium-treated and control rats restricted to 15 ml of water per day.
Rats were treated as described in figures 18 and 23. Plasma alkaline phosphatase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) of activity = 1μmole *p*-nitrophenol/hr.

In rats offered water *ad libitum*, active (exercise-induced) heating increased plasma alanine aminotransferase (ALT) activity in lithium treated and control rats (figure 29; $p< 0.05$ different from no heat by 1-way ANOVA and Scheffe's test). There was no

difference between lithium treated and control rats. Passive heating had no significant effect upon ALT in unrestricted fluid rats (by 1-way ANOVA and Scheffe's test). In rats restricted to 15 ml water, neither active (exercise-induced) or passive heating increased plasma ALT activity significantly (figure 30; by 1-way ANOVA and Scheffe's test; note the high value measured in passive lithium group occurred because 2/5 rats had extremely high ALT, when analyzed by 1-way ANOVA and Scheffe's test p approaches but does not achieve 0.05 level).

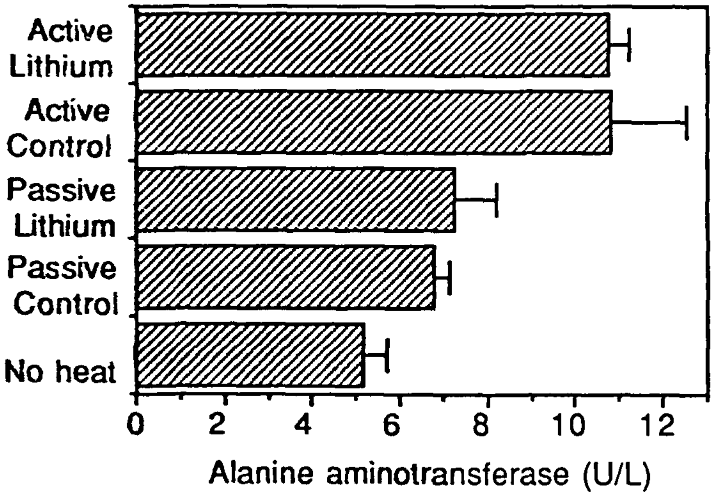


Fig 29: The effect of heating upon plasma alanine aminotransferase activities in lithium-treated and control rats offered water ad lib.

Rats were treated as described in figures 15 and 19. Plasma alanine aminotransferase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) produces 1 μmole NAD/minute.

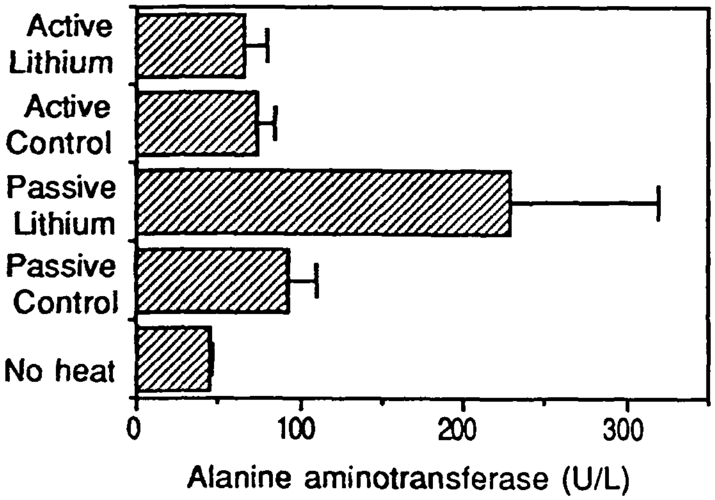


Fig 30: The effect of heating upon plasma alanine aminotransferase activities in lithium-treated and control rats restricted to 15 ml of water per day.

Rats were treated as described in figures 18 and 23. Plasma alanine aminotransferase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) produces 1 μmole NAD/minute.

In rats offered water *ad libitum*, active (exercise-induced) heating increased plasma lactic dehydrogenase (LDH) activity (figure 31; $p<0.01$ different from no heat by 1-way ANOVA and Scheffe's test) in the lithium treated group (the control group tended to increase, but no significant difference from no heat by 1-way ANOVA and Scheffe's test). Passive heating of unrestricted rats did not elevate LDH activity in either the lithium treated or control groups. In the 15 ml fluid restricted rats there were no significant differences in LDH activity between the lithium treated and control rats (by 1-way ANOVA and Scheffe's test; figure 32). Passive heating of fluid restricted rats significantly elevated plasma LDH

activity (both groups p<0.01 different from no heat by 1-way ANOVA and Scheffe's test). Active (exercise-induced) heating of fluid restricted rats did not elevate plasma LDH activity.

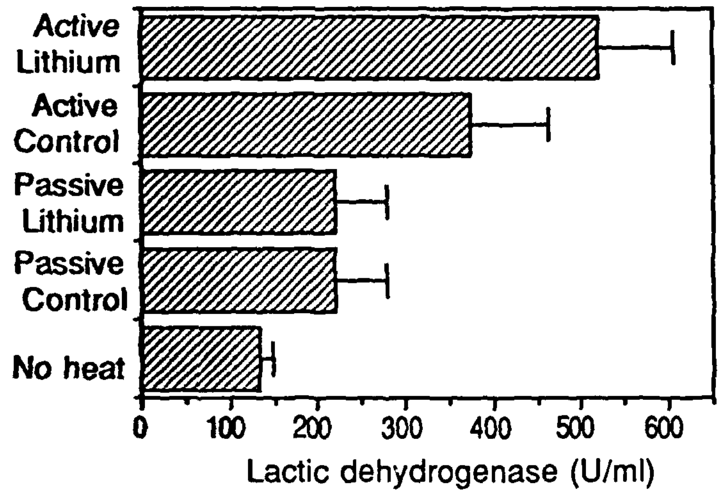


Fig 31: The effect of heating upon plasma lactic dehydrogenase activities in lithium-treated and control rats offered water ad lib.
Rats were treated as described in figures 15 and 19. Plasma lactic dehydrogenase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) converts 1 μmole of substrate/minute.

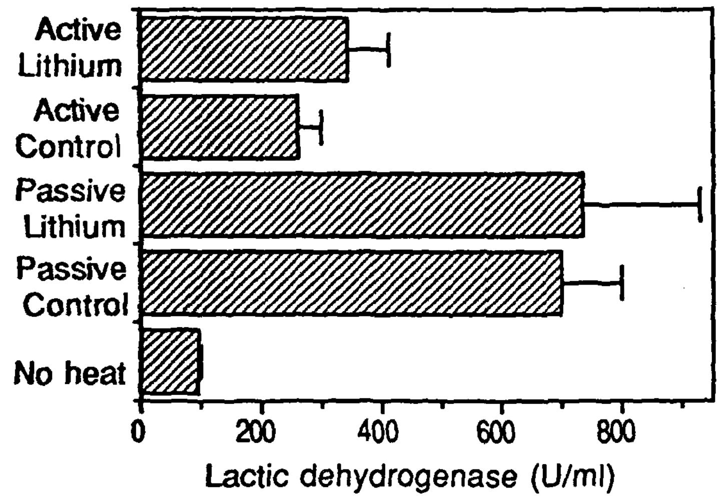


Fig 32: The effect of heating upon lactic dehydrogenase activities in lithium-treated and control rats restricted to 15 ml of water per day.
Rats were treated as described in figures 18 and 23. Plasma lactic dehydrogenase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) converts 1 μmole of substrate/minute.

In rats offered water *ad libitum*, active (exercise-induced) heating of lithium treated increased plasma creatine phosphokinase activity (CPK; figure 33 p <0.05 different from no heat by 1-way ANOVA and Scheffe's test) and tended to increase activity in control animals (no difference between lithium treated and control active (exercise-induced) heated). Passive heating did not elevate CPK in any of the groups of unrestricted rats.

In the 15 ml restricted rats, passive heating significantly elevated CPK activity in the lithium treated animals (figure 34; p <0.05 different from no heat by 1-way ANOVA and Scheffe's test). The difference between passively heated lithium treated and control animals was not significant (by 1-way ANOVA and Scheffe's test).

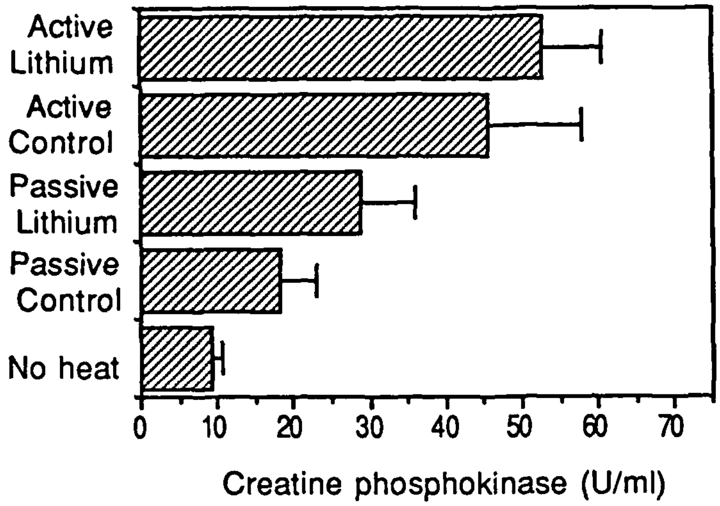


Fig 33: The effect of heating upon plasma creatine phosphokinase activities in lithium-treated and control rats offered water ad lib.

Rats were treated as described in figures 15 and 19. Plasma creatine phosphokinase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) phosphorylates 1 nmole of creatine/minute.

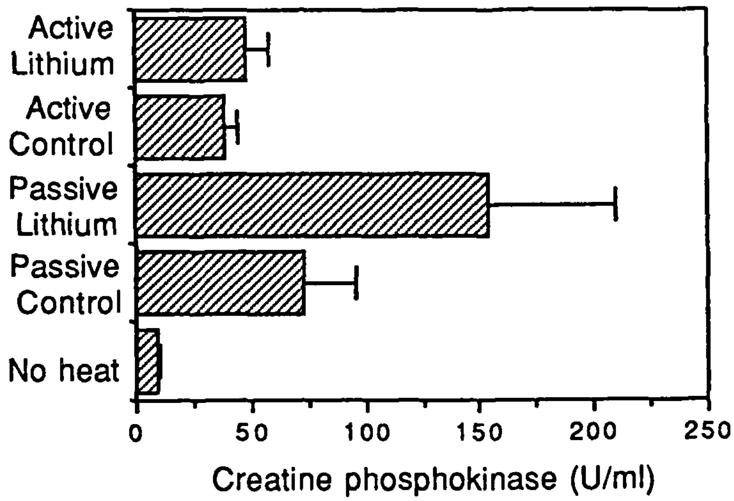


Fig 34: The effect of heating upon creatine phosphokinase activities in lithium-treated and control rats restricted to 15 ml of water per day.

Rats were treated as described in figures 18 and 23. Plasma creatine phosphokinase activity was determined as described in the Methods section. Data are presented as mean ± standard error of the mean. One unit (U) phosphorylates 1 nmole of creatine/minute.

Conclusions:

The lithium levels achieved by our treatments (figures 9 and 10) are similar to those achieved in humans being treated for psychiatric disorders (0.5-1 mM). These doses caused no toxicity in the rats, and animals gained weight at the same rate as did controls (figures 2-8).

Lithium treatment did not significantly alter the response of rats to heating using the passive or active (exercise-induced) heating models (figures 15-26; tables 2-7 and 9-10). As animals were passively heated, core temperatures rose at similar rates in both control and matched treatment groups (table 9). Lithium treated animals also lost similar (compared to matched controls) amounts of water during passive and active heating (table 8-10). During active (exercise-induced) heating, changes in body temperatures were similar between lithium treated and matched control rats during exercise (figures 19-25, tables 6-7 and 10), but tail temperatures for ad lib fluid rats were consistently higher in lithium treated rats during cooling (figure 22) but this was not so for the fluid restricted groups (figure 26). A similar trend (not statistically significant) was observed for core body temperature cooling rates - temperatures for ad lib fluid rats were consistently higher in lithium treated rats during cooling (figure 21) but this was not so for the fluid restricted groups (figure 25). This data supports the hypothesis that lithium treatment changes the rats' ability to cool themselves, we speculate that this may reflect a difference in control of vasodilation.

The major effects that we observed were associated with fluid restriction rather than with lithium treatment. Total body water, extracellular fluid volumes and plasma volumes were decreased in fluid restricted versus *ad libitum* rats (figures 11-14, table 1). The decrease in plasma volume secondary to fluid restriction tended to be smaller in lithium treated than in control animals (5.7 versus 3.1 ml decrease; table 1; not significantly different). Fluid restricted animals lost less weight during heating (table 8) and lost this weight less rapidly during heating (tables 9 and 10). This probably reflects their diminished fluid status at the start of the experiments, and the shorter times required to achieve 42.6°C for passive heating (table 9). However, for the active (exercise-induced) heating, maximum temperature achieved and heating rate were not increased in the fluid restricted rats (table 10), in fact fluid restricted control animals took longer to reach exhaustion than did ad lib control rats (table 10). Degree-minutes above 40.5°C was not changed by lithium treatment, and was diminished slightly by fluid restriction (table 11). This is difficult to understand and should be replicated before definite conclusions are reached.

The increased heating rates (tables 9 and 10) and decreased times required to achieve heating criteria (for the passive heating experiments) in fluid restricted rats probably occurred because the rats' ability to cool themselves was impaired (figures 25 versus 26). Rats cool themselves by increasing blood flow to the tail (23) and by secreting copious amounts of saliva and excreting urine, with which they wet skin surface and thereby lose heat by evaporation (23). Dehydration would be expected to impair these methods for cooling. For running animals skin wetting is impossible (they can not gather fluid and wet skin), and therefore these mechanisms for cooling are less important - this may explain why fluid restriction was not associated with a change in time to reach exhaustion or in heating rates (table 10). We have no explanation for why fluid restriction lowered maximum core body temperature achieved prior to exhaustion (table 10).

Organ damage, was assessed by measuring leakage of ALT, LDH, CPK and alkaline phosphatase into blood. ALT and LDH are markers for liver damage, CPK for muscle damage, and alkaline phosphatase for liver, bone or intestinal damage. Alkaline phosphatase was elevated in all ad libitum water groups that were heated (figure 27). It is a very easily released enzyme (38), and therefore is an extremely sensitive marker. In fluid restricted rats alkaline phosphatase was only elevated in actively heated animals (figure 28). Perhaps, for passive heating to release alkaline phosphatase prolonged exposure to heat is

required. Passively heated ad lib water rats took approximately 250 minutes to reach 42.6°C while fluid restricted rats took only approximately 150 minutes (tables 9 and 10).

ALT was elevated in all heated animals offered unrestricted water, though more so in the actively heated rats (figure 31). These elevations were not observed in fluid restricted rats (figure 30) that were actively heated. Perhaps this occurred because maximum core body temperature achieved was lower in these 2 groups (table 10), or because degree-minutes above 40.5°C were fewer (table 11). This diminishing of time of exposure to high temperature is an interesting phenomenon, and these results should be confirmed. We have no explanation for why ALT was elevated in fluid restricted rats treated with lithium and passively heated.

LDH was elevated in all heated animals (figures 31 and 32). In passively heated animals LDH was much more elevated in fluid restricted animals. Restricted rats which were passively heated warmed up much faster than did all other groups (0.04°/min versus 0.02°/min). Perhaps LDH release is increased during rapid heating. The dissociation between LDH and ALT data that we observed suggests that LDH may have been released from a tissue other than liver. It is also possible that different amounts of cell damage are required for LDH versus ALT leakage.

CPK was elevated in all heated animals offered unrestricted water, though more so in the actively heated rats (figure 33). These elevations were not as great in fluid restricted rats (figure 34) that were actively heated. Why did fluid restriction diminish the rise in serum CPK associated with active heating? Perhaps this occurred because maximum core body temperature achieved was lower in these 2 groups (table 10). This is a pattern similar to that which we observed for ALT. Passively heated fluid restricted rats did have increased CPK (figure 34). Again a pattern similar to that observed for ALT. The ALT and CPK data suggest that during exercise induced heating core temperatures must exceed a threshold (higher than 40.73°C) before cells are damaged enough to release these enzymes. Hubbard *et al.* (21) observed that CPK release was associated with duration of exercise rather than with degree of hyperthermia. In our studies the group which endured exertion the longest (control restricted rats; table 10) did not have the highest CPK (figure 34).

We observed that lithium treated animals consistently drank more when offered water *ad libitum* (figure 8). This group drank more than 70 ml/day once plasma lithium levels were elevated (figures 8 and 10). Control animals drank 40-50 ml/day (figure 8). This intake in controls is normal for 350-400 gram rats eating a pelleted diet (normal range is 80-110 ml/kg body weight/day = 44 ml/day in a 400 gram rat, and this varies with the dryness of the diet; 39). An artifacts due to spillage/evaporation would equally influence data in control and lithium groups. Lithium treatment in humans has often been associated with polyuria (10,16) thought to be secondary to renal toxicity of lithium. Increased loss of fluid in urine would necessitate increased drinking of water to maintain body water pools. In an attempt to determine whether renal toxicity of lithium acted to increase water demand in rats, we restricted the availability of water and determined whether lithium treated rats could regulate body water distribution. We found that even when water intake was severely restricted (to 15 ml/day; resulted in weight loss and dehydration in all groups), lithium treated animals had the same body water distribution as did controls restricted in the same way (figures 11-14, table 1). Thus, lithium treated animals were able to regulate body water distribution and to conserve water in the face of severe restriction. Probably the increased ingestion of fluid in *ad libitum* water-lithium treated animals occurred because of increased thirst, rather than because of an obligatory loss of fluid in the urine.

In summary, the data collected supports the hypothesis that lithium treatment changes the rats' ability to cool themselves after they had been actively heated, we speculate that this may reflect a difference in control of vasodilation in the tail. This effect did not appreciably alter heating rates. We did not observe any effect of lithium upon body water distribution, or upon weight loss during heating. We did observe that fluid restriction increased rate of heating in a model in which salivation and urination are important for

cooling. Salivation and urination might have been diminished because, in these animals, there was decreased total body water, extracellular fluid and plasma volume. These findings are consistent with those previously reported. Our enzyme leakage data suggest that cell damage is influenced by maximaum temperature achieved, rate of heating and duration of heating. Different tissues (enzymes) may be differently affected by these variables.

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One manuscript has been published:

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